UNIVERSITI PUTRA MALAYSIA

CYTOGENETIC STUDIES ON THE WATER BUFFALO (*Bubalus bubalis*)

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CYTOGENETIC STUDIES ON THE

WATER BUFFALO *(Bubalus bubalis)*

by

MOHAMAD HILMI BIN HAJI ABDULLAH

A Thesis

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This thesis is dedicated to

my parents, wife and children

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Chromosome analysis was undertaken on Murrah, swamp, F_1 (Murrah x swamp) and backcross (F_1 female x Murrah male) buffaloes *(Bubalus bubalis)* using a modified leucocyte culture method combined with Giemsa (G) constitutive heterochromatin (C) and nucleolus organiser region (NOR) banding procedures. A combination of 80% medium RPMI 1640, 20% calf serum, pokeweed mitogen and buffy coat gave many good quality metaphases. The Murrah, swamp and F_1 buffaloes had chromosome complements of 2n = 50, 2n = 48 and 2n = 49 respectively. The Murrah karyotype had 5 pairs of submetacentric chromosomes and 20 pairs of acrocentric chromosomes while the swamp karyotype had 1 pair of large metacentric chromosomes, 4

submetacentric pairs and 19 pairs of acrocentric chromosomes. F_1 hybrid had an intermediate karyotype. Nine backcross animals had a 2n = 49 complement while 3 backcross animals had a 2n = 50 complement. Based on banding patterns (G, C and NOR), chromatid arm ratios and chromosome size it was revealed that the largest two metacentric chromosomes of the swamp buffalo resulted from a telomere - centromere tandem fusion between chromosomes 4p and 9 of the Murrah karyotype. This 4/9 tandem fusion was probably responsible for the evolutionary dichotomy of this species. The acrocentric X chromosomes had large triangular masses of constitutive heterochromatin, one pair of dark bands in the chromatids proximal to centromere and distinct telomeric bands. The Y chromosome had distinct telomeric bands, no C band and was not the smallest chromosome in the buffalo karyotype.

Testicular biopsies revealed all the meiotic stages in Murrah, swamp and F_1 buffaloes. However, chromosome sets from 22 to 26 (most frequent 24 and 25) with many cells carrying univalent, bivalent and multivalent configurations were observed in F_1 hybrids, while the Murrah and swamp showed chromosome sets of 25 and 24 bivalents respectively. Degenerating spermatocytes and abnormal spermatids were observed on histological and electron microscopic examinations of hybrid testes suggesting that various synaptic associations leading to unbalanced gametes may be responsible for the degenerating germ cells. Because of a large percentage of germinal epithelial cells being wasted in F_1 hybrids it is suggested that fertility of backcross and F_2 generation may be subnormal.

INTRODUCTION

The water buffalo *(Bubalus bubalis)* is becoming increasingly important as a source of milk, meat and draft in many tropical, subtropical and warm temperate zones in developing and developed countries. The water buffalo has been considered a symbol of Asian life and endurance and is performing well in research trials in U.S.A., Papua New Guinea, Trinidad, Costa Rica, Venezuela, Brazil, Italy and Egypt. Out of a total world population of 130 million water buffaloes, approximately 116 million exist in Asia (Mahadevan, 1979). Because of its resistance to disease, ability to convert poor quality roughage to meat and milk and its powerful draft capacity, it has become an important animal in the agricultural economy of most Asian countries.

Water buffaloes have been classified into the river and swamp types. The river type is larger, used for milk, wallows in fresh-water and originates mostly in the Indian subcontinent and the Near East. The swamp type is smaller, used for draft and meat, wallows in muddy water and is indigenous to most Asian countries (Mason, 1974; Cockrill, 1981). In Malaysia, approximately 50% of the beef supply comes from the water buffalo (Syed Ali Bakar, 1980) and this animal is being increasingly used as a source of draft to carry oil-palm bunches in estates where the foliage prevents the use of machinery. In view of its many uses and sharp decline there has been tremendous interest in increasing its productivity through organised research. One of the research priority areas identified by the Food and Agricultural Organisation (FAO) has been in the area of genetic improvement (Mahadevan, 1977). In category II of this report an international cooperative testing programme was urgently required to determine the relative genetic merits of different breeds and strains of water buffalo. Further, results of crossing of principal strains and evaluation of merits of first crosses and backcross to the paternal strains was also badly needed so as to improve buffalo productivity.

Water buffaloes in Asia can also be divided according to cytogenetic status. The chromosome complements of the swamp buffalo of Asia and Australia have been reported to be 2n = 48 (Ulbrich and Fischer, 1967; Toll and Halnan, 1976) while the river types carried 2n = 50 chromosome complements (Fischer 1971). The indigenous buffalo of Sri Lanka traditionally classified as the swamp type, carried 2n = 50 chromosomes (Scheurmann, *et al.*, 1974; Bongso *et al.*, 1977). The smaller swamp types of Asia are being upgraded with the river types (e.g. Murrah) imported from India so as to reap the advantages of hybrid vigour. The resulting hybrid has exhibited better productivity in the form of better growth rates and increased carcase yields as compared to the swamp type. While the advantages of heterosis have been observed, the implications of differences in chromosome number in the parents have not been investigated. Recently, it has been shown that the chromosome complement of the F_1 hybrid (river x swamp) buffalo was 2n = 49, with a karyotype intermediate to the two parental types (Bongso and Jainudeen, 1979).

Reproductive fitness has been shown to be affected in other hybrids possesing chromosome complements different from parental types, such as the mule and hinny (Benirschke 1967; Chandley, *et al.*, 1974), zebra x donkey (King 1967) cattalo (Basrur, 1969) and Indian x

Chinese muntjac (Shi Liming and Pathak, 1981). In all these hybrids chromosome non-homology in the parental types was incriminated as the main cause of infertility. Thus, in order to relate chromosome rearrangements with fertility, studies were undertaken to (1) first establish a suitable culture technique for the water buffalo and investigate the detailed karyotype (number and morphology) of the Murrah and swamp buffaloes and their crossbreds using conventional, giemsa (G), constitutive heterochromatin (C) and nucleolus organizer region (NOR) banding patterns (2) identify the chromosomes responsible for the evolutionary dichotomy of this species and (3) investigate the seggregation of chromosomes during meiosis in F_1 hybrid males and backcrosses so as to provide information as to the effect of the 2n=49 chromosome complement on fertility.

From the results obtained it was hoped that a sufficient base could be developed to help formulate an appropriate breeding policy aimed at increasing productivity for the water buffalo in Asia. These results would also provide answers to category II of the FAO technical advisory committee report on the genetic merits of crossbreeding in the water buffalo.

LITERATURE REVIEW

Zoological Position of Water Buffalo

Inspite of new development in the zoological systematics in recent years and a mass of biometrical data on anatomical and genetical features of the domestic animals, there is yet no clearcut and universally accepted classification of both, wild and domestic buffaloes. Various classifications of the water buffalo have been reported (Simpson, 1945; Bohlken, 1958; Mason, 1974; Fahimuddin, 1975) and these have been summarised in Figure 1.

Two major groups of buffaloes exist *viz.*, the Syncerina comprising African buffaloes and the Bubalina, made up of Asian buffaloes. There are fundamental anatomical differences justifying their separation into these two groups. The Asian buffalo comes within the genus *Bubalus*. In the Pleistocene period, the genus *Bubalus* was widely distributed in Europe and Southern Asia. There are three main wild buffalo species within Asia which are distinct and have thus attracted individual names. These are the Anoa of the Celebes island of Indonesia, the Tamarao in the island of Mindoro in the Philippines and the Arni or Indian wild buffalo which is now domesticated (Mason, 1974).

Bohlken (1958) considered the Anoa to be only specifically different from other Asian buffaloes and thus called it *Bubalus depressicornis*. But because of its small size as compared to other buffaloes, and short horns pointing straight backwards it has been given the zoological name *Anoa depressicornis* (Mason, 1974). The Anoa has been divided into two subspecies differing in size, colour

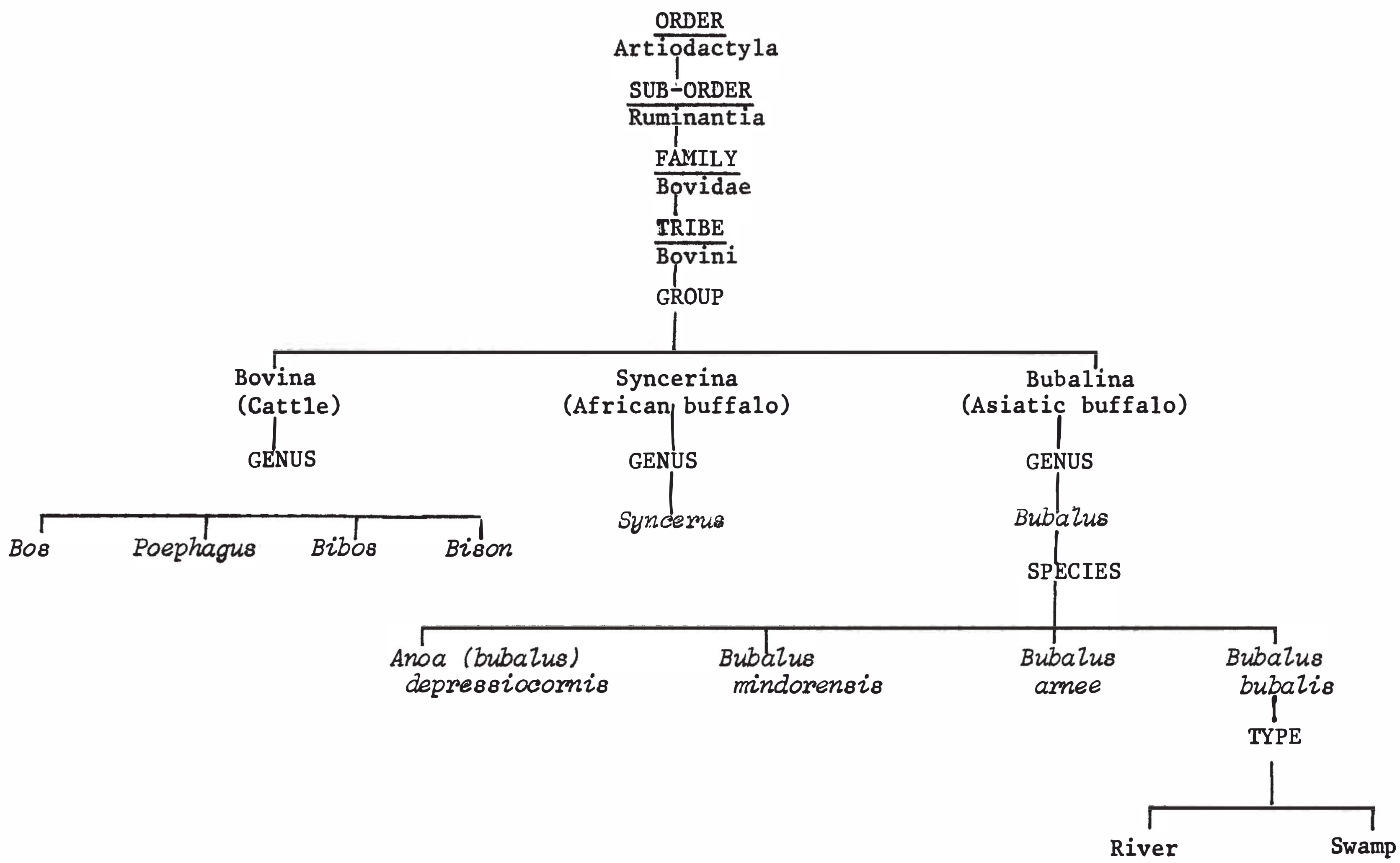

Figure 1. Zoological Position of Water Buffalo

and habitat. These are the common or lowland Anoa *(Bubalus depressicornis depressicornis)* and small or mountain Anoa *(Bubalus depressicornis quarlesi)*.

The Tamarao is commonly referred to as the Mindoro buffalo and classified as *Bubalus mindorensis*. It is in many ways, intermediate to the Anoa and Arni being a small animal, grey black or dark brown, white marks on head, neck and legs and short, strong horns with points turned inwards (Mason, 1974).

The Indian wild buffalo received its name from the Hindi arni and is referred to as *Bubalus arnee*. Its present distribution is in Northern India and Sri Lanka. It is a very large animal, grey-black in colour and with distinct white chevron markings on the neck and brisket. It has large horns widely separated at the base and curve round in a sickle shape with tips pointing inwards (Mason, 1974). The exact status of the Sri Lanka wild buffalo is controversial. Deraniyagala (1953-1955) classified it as a separate subspecies differing from the Arni in its smaller size and shorter horns curving further forward at the tip. However, Ellerman and Morrison-Scott (1951) dismissed the Sri Lanka buffalo as probably feral. Leupold (1968) reported that they are similar to swamp buffaloes. It has been claimed that Sri Lanka had no wild buffaloes, and that the wild types are feral animals escaped from captivity. They have been derived from Indian stock which is mainly of the river type. The Sri Lanka buffalo cannot be regarded as swamp type (Fahimuddin, 1975). This author also categorically states that the swamp type of Sri Lanka appears to be a degenerate type of the river buffalo. It is interesting to note that recent studies on the chromosome makeup of the swamp buffalo of Sri Lanka showed that it had 50 chromosomes, similar to the chromosome number observed in the river types (Scheurmann *et al.*, 1974, Bongso *et al.*, 1977). Based on chromosome studies, Bongso *et al.*,(1977) suggested that the Sri Lankan buffalo probably originated from the Indian river type and in the course of time, when man's emphasis on the animal's use shifted from milk to draft in marshy lands, it lost its dairy characteristics and acquired swamp habits.

The zoological classification of the present day domestic buffalo is not clear-cut. Based on Linnaeus's terminology, it comprises a separate species *Bubalus bubalis* and according to Bohlken's terminology it is a sub-species and should be called *Bubalus arnee* forma *bubalis*. However, current literature refers to the domestic buffalo as *Bubalus bubalis*.

Breeds and Types of Domestic Buffaloes

MacGregor (1939) classified the domestic buffalo into river and swamp types based on habitat and use under domestication. The swamp type prefers the swamp or marshland and is used mainly for draft or meat while the river type prefers clean water of rivers and is primarily used for milk.

The swamp buffalo found mainly in Southeast Asia extends northward as far as the Yangtze valley in China and westward as far as Assam (Mason, 1974). The swamp buffaloes in different areas have been given local names and it is thus misleading to call them independent breeds. In Malaysia, the swamp type has been given the general indigenous name of *kerbau sawah* and the river type called *kerbau sapi*. Ten percent of Malaysia's swamp buffalo population is albinoid and referred to as *kerbau balar*. These animals are pink in colour with black freckling but do not have the typical eye and conjunctiva colour described for albinos. In the Philippines the term carabao has been given to the indigenous swamp buffalo and recently it has been suggested that this variety should be reclassified as *Bubalus carabanensis* (Linn.) (Castillo, 1971). The swamp types of other countries have no special names but referred to after their country of origin.

The river type of buffalo is mainly confined to India and Pakistan. They have a distinctly different phenotype from the swamp types and have definite breeds belonging to groups *viz.*, Murrah group, Gujarat, Uttar Pradesh, Central India varieties and South Indian varieties (Mason, 1974). The Murrah and Surti breeds belong to the Murrah and Gujarat groups respectively. These are the two major breeds exported to Southeast Asian countries to upgrade the indigenous swamp varieties by crossbreeding.

The river types have also been introduced in the Near East, Europe, Australia, Latin America and Africa and although river in terms of type, they have attained phenotypic characteristics different from the Murrah and Surti breeds of India (Cockrill, 1981).

The buffaloes of Yugoslavia and other European countries have been referred to as the Mediterranean type of river buffalo by MacGregor (1941), because they originated from the Mediterrnean basin.

World Distribution

The distribution of water buffaloes in the world are shown in Table I. It is quite interesting to note that approximately 88 percent of water buffaloes are in the Asian region. The swamp type is confined mainly to southeast China, Burma, Assam, Laos, the Khmer Republic, Vietnam, Thailand, Malaysia, Indonesia, the Philippines and Sri Lanka. The river types are found mainly in India and Pakistan and introduced to Oceania, Iran, Iraq, Afghanistan, Turkey, Egypt, Africa, Latin America and Europe. Many buffaloes of Northern Nepal and Southern India are of the swamp type. The approximate proportions of river, swamp and Mediterranean type buffaloes are 66.7%, 29.7% and 3.6% respectively (Mahadevan, 1983). The numbers of buffaloes in countries with sizeable populations of the swamp type are shown in Table II.

River, Swamp and Crossbred Buffaloes of Malaysia

In most Southeast Asian countries the indigenous swamp varieties are being upgraded by crossing them with imported larger river types, such as the Murrah breed so as to reap the advantages of hybrid vigour for better growth rate and carcase yield. This is particularly so in Malaysia because approximately 50% of the beef supply from the livestock industry comes from the water buffalo.

In Malaysia, inspite of the introduction of farm machinery, buffaloes are still being used for work in certain locations such as, deep-water rice areas and small-holdings for hauling and transporting rice during harvest, carting oil palm bunches in estates and carting of fish. In the single cropping rice areas the

Table 1. World distribution of water buffaloes

Country		Buffalo Population
ASIA	: Burma	1,883,000
	China	30,110,000
	India	60,767,000
	Indonesia	2,823,000
	Laos	1,072,000
	Malaysia	285,000
	Philippines	3,860,000
	Sri Lanka	736,000
	Thailand	5,784,000
	Vietnam	2,330,000
	Others	2,238,000
EUROPE	: Bulgaria	68,000
	Italy	81,000
	Romania	206,000
	Others	78,000
NEAR EAST	: Egypt	2,280,000
	Iran	130,000
	Iraq	332,000
	Pakistan	10,563,000
	Turkey	1,022,000
	USSR	472,000
OCEANIA	: Australia	200,000
OTHERS	: South America	166,000
	Carribean	7,000
	World Total	132,498,000

Source: Mahadevan, 1979

Table II. Countries with sizeable populations of swamp buffalo type

Country	Thousand head
Burma	1950
China	18520
Indonesia	2488
Kampuchea	404
Laos	880
Malaysia	2782
Philippines	6124
Vietnam	2378
Total	35819

Source: Mahadevan (1983)